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RESEARCH ARTICLE

Differential expression of Oman's wild lavender, lavandula subnuda for chemical composition towards medicinal and aromatic application

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ABSTRACT

Plants have been used directly or indirectly as medicines for over 5000 years as a source of antibiotics, antineoplastics, analgesics, and cardio-protective, among others. Approximately 70–90% of the population in developing countries continue to use ancient medicines based on plant extracts for treatment. Recently, the isolation and identification of biologically active compounds and molecules from nature have led to the discovery of new therapeutics, prompting the improvement of the health and pharmaceutical sectors. Phytochemicals revolve around the pharmaceutical industry's research and development (R&D) sector as a source of new molecules leading to the development of new novel drugs. Given the above, the present investigation addresses the differential nature of wild lavender (*Lavandula subnuda* Benth) plants of diverse locations in Oman with respect to their chemical composition of the essential oil in addition to morphological characters and chlorophyll contents of leaves to explore the possibility of isolating its prime chemical compounds on a commercial scale in perfumery industry. There are no previous studies so far who have reported on essential oil recovery and chemical composition exclusively in respect of *Lavandula subnuda*. Composite samples of ten randomly selected plants were collected from wadi habitats of four diversified locations. Edaphic features of managed sites were recorded, and their soil chemical contents were determined following instructions using S1 Titan/Tracer 5/CTX equipment of Bruker developed based on energy dispersive X-ray fluorescence (EDXRF). Morphological traits were measured using a ruler, and chlorophyll contents were recorded using the atLEAF CHL Plus chlorophyll meter as atLEAF values. The essential oil was extracted using ETHO X's advanced microwave extraction system and analyzed for chemical compounds using GC-MS analysis on Shimadzu GC-2010 Plus gas chromatograph. The experimental data were analyzed statistically, wherever required, by applying basic statistics of the EXCEL -16 version. The results indicated that in general, the *Lavandula subnuda* plants of locations of high altitudes had higher expressivity in all the morphological traits and chlorophyll contents than those of low altitudes. The essential oil recovery, however, was found to be higher from the plant samples of lower altitudes (0.70 to 0.79% (w/w)) than from those of higher mountains/altitudes (0.15 to 0.18% (w/w)). The pattern of values of commonly occurring chemical contents of essential oils was different. Each essential oil's top ten chemical compounds contributed about 80% of the total in four locations. Of the ten top chemical compounds, eight compounds, namely D-Germacrene (42.67%) from Wadi Al-Khod, Estragole (32%) and Linalool (23.89%) from Wadi Halban), trans-Borneol (23.46%) and 4-Terpineol (18.73%) from Wadi Najd Al-Waqba and Kessane (18.60%), beta-cis-Caryophyllene (13.68%) and beta-Elemene (10.618%) from Wadi Al-Hayul, were found highest in quantity and had huge potential for further application. It is concluded that there exists a higher possibility of adapting Good Agriculture Practice (GAP) to produce the highest herbage yield of vegetative parts of *Lavandula subnuda* plants based on morphological features under wild conditions to extract these compounds on a commercial scale in both the pharmaceutical and perfumery industries.

Keywords: Morphology; Chlorophyll; Chemical compounds; Differential Expression; *Lavandula subnuda*; Lamiaceae

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INTRODUCTION

The traditional medicine practices are now in vogue for health care in most countries of the world owing to their derivation from indigenous knowledge and least or no side effects (Barata et al., 2016). It is expected that eighty percent of the world population primarily rely on plant-based drugs for their healthcare needs by the mid-2020s (FAO, 2005; Barata et al., 2016; Anand et al., 2019). Natural medicinal products are recommended for their use as either complementary or alternative to allopathic medicine for treating several ailments like cancer, diabetes, and blood pressure in human beings (Sofowora et al., 2013; Hassan-Abdallah et al., 2013; Santic et al., 2017; Zivkovic et al., 2020; Amin et al., 2021). It has been estimated that around 60,000 species are used worldwide for their medicinal and aromatic properties, and more than 500,000 tons of materials are traded (WHO, 2015).

A report from the Royal Botanic Gardens, Kew, the United Kingdom, shows that about 369,000 species (or 94 percent) are flowering plants worldwide (Dasgupta, 2016). The Sultanate of Oman has physiographic and climatic features of African and Asian continents that harbor all species, from typical temperate and subtropical to tropical crops, herbs, grasses, shrubs to tree plants. The "Socioeconomic Plants Conservation Strategy for the Sultanate of Oman", developed by the Oman Animal and Plant Genetic Resources Center (OAPGRC) of the Ministry of Higher Education, Research and Innovation, reports that Oman has about 1578 flowering plants, of which 448 medicinal plant species from 283 genera and 95 families are either medicinal, aromatic or both (Al Lawati et al., 2017).

The genus Lavandula (Lamiaceae) consists of about 47 species of flowering plants in the Lamiaceae family and is native to the Old-World countries and is found from Cape Verde and the Canary Islands, Europe across northern and eastern Africa, the Mediterranean, southwest Asia to the Indian subcontinent as both medicinal and aromatic plants as well as ornamental (Wikipedia, 2022). Lavender species in general (Smigielski et al., 2009; Danh et al., 2013; Jianu et al., 2013) including Lavandula subnuda Benth are known to have both medicinal and aromatic properties (Messaoud et al., 2012; Anonymous, 2022). These species are wildly available in wadi habitats across all the governorates of Oman. Worldwide lavender is used not only in pharmaceutical and perfumery but also in cosmetics and soap industries. In the Middle East countries including Oman, it is used reported for its use as repellent against moths and insects and as stimulant, tonic and antispasmodic. It is also known for its use for its treatment against the bites of dogs and snakes. In general all the parts viz. stems, leaves and flowers of the plants are used (Miller and Morris, 1988).

Soil properties are reported to have a significant role not only in the growth and development of the plant but also in the chemical composition of active substances like essential oils (Ferraz et al., 2018; Hendawy et al., 2019). Soil chemical elements such as nitrogen, phosphorus, and potassium would involve enzymes in the biochemical processes of the plants and are influenced by environmental factors, including soil features and elevations (Feng et al., 2021; Guedri Mkaddem et al., 2022). These affect the yield and composition of essential oils (Yavari et al., 2010; Vaičiulytė et al., 2016). In addition, the chemical composition of essential oils varies depending on agro-climatological conditions where plants grow (Nteziyaremye et al., 2021). There have no studies reported so far on essential oil yields (%) and chemical composition of *Lavandula subnuda* under varying edaphic and climatic conditions. Hence, we undertook the present investigation to study the differential nature of wild lavender (Lavandula subnuda) plants to four locations of Oman from where from plant samples were collected in respect of growth and chemical composition for their application in medicinal and aromatic industries.

MATERIALS AND METHODS

Plant material

The composite samples of both leaves and stems of Vitex agnus castus L. were collected during the Winter of 2021 from four wadi habitats of four different governorates of Oman. These were Al-Khoud of Wilayat Al-Seeb of Muscat governorate (58.110° E; 23.558° N) located at the altitude of 100 m; Halban of Wilayat Barka of South Batinah governorate (58.041°E; 23.319°N) situated at 96 m; Najd Al-Waqba of Wilayat Yankul of Al-Dhairah governorate (56.416° E; 23.898° N) located in the higher mountain at the altitude of 758 m and Al-Hayul of Wilayat Mahdha of Al-Buraimi governorate (56.318o E; 23.372o N) situated in the high peak at 544 m) (Fig. 1; Plate 1 (a-f); Table 1). At the same time, the relevant edaphic features of locations, such as soil type, structure, critical chemical contents, soil pH and salinity, and morphological characters of 10 randomly selected plants, were recorded. The voucher specimens of *Lavandula subnuda* Boiss were collected and submitted to the Life Science Unit of Sultan Qaboos University for confirmation by the taxonomist at the Sultan Qaboos University Herbarium (SPUH). The voucher material was then deposited in the herbarium collection under barcode SQUH00006294 (https://herb.squ.edu.om/view_specimen/SmZkL2Y0NjFhZlhMdTAxT0VYVnY4Zz09). The requisite plant samples were shade-dried before their use for the extraction of essential oil and further chemical analyses.

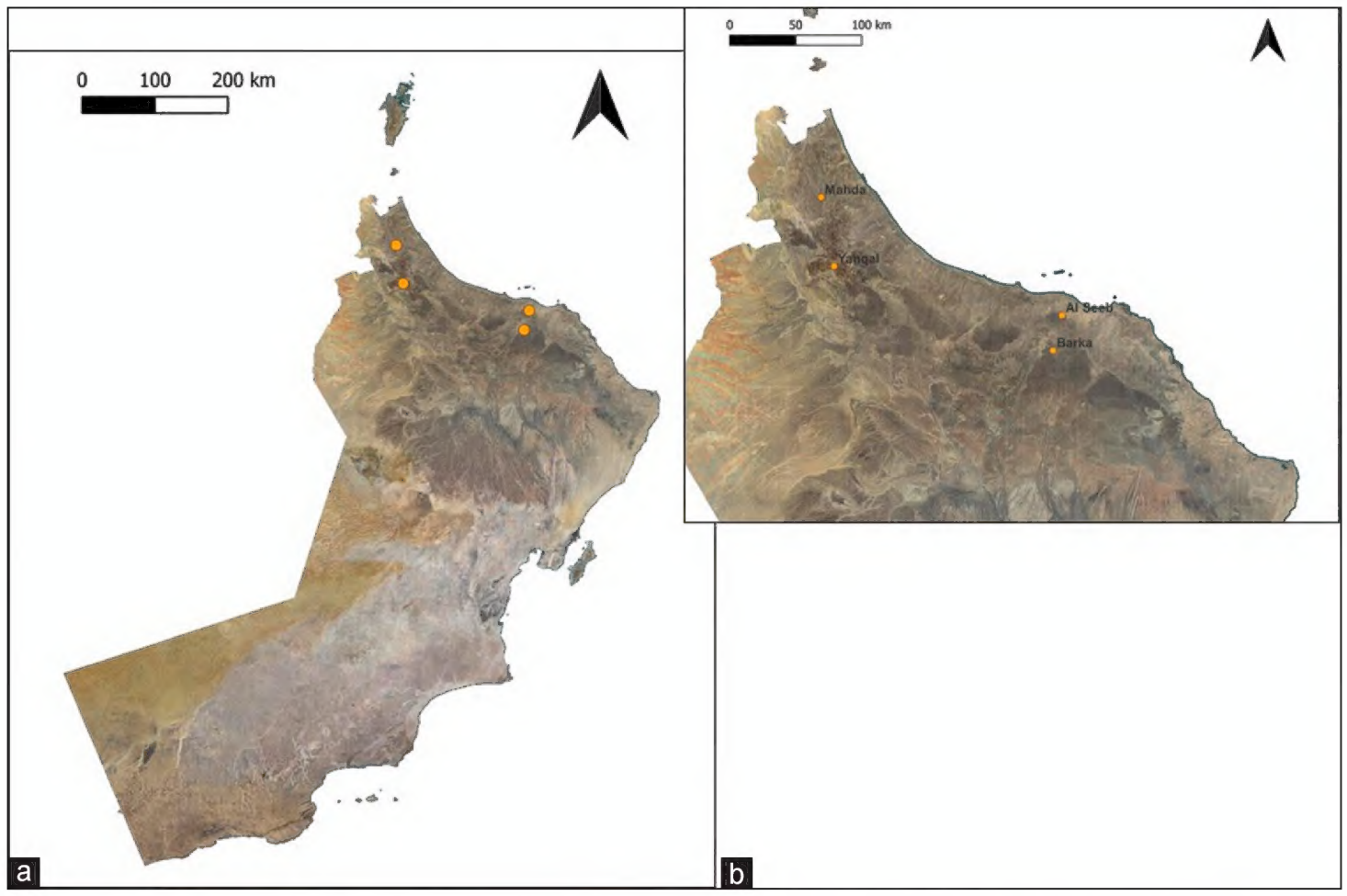
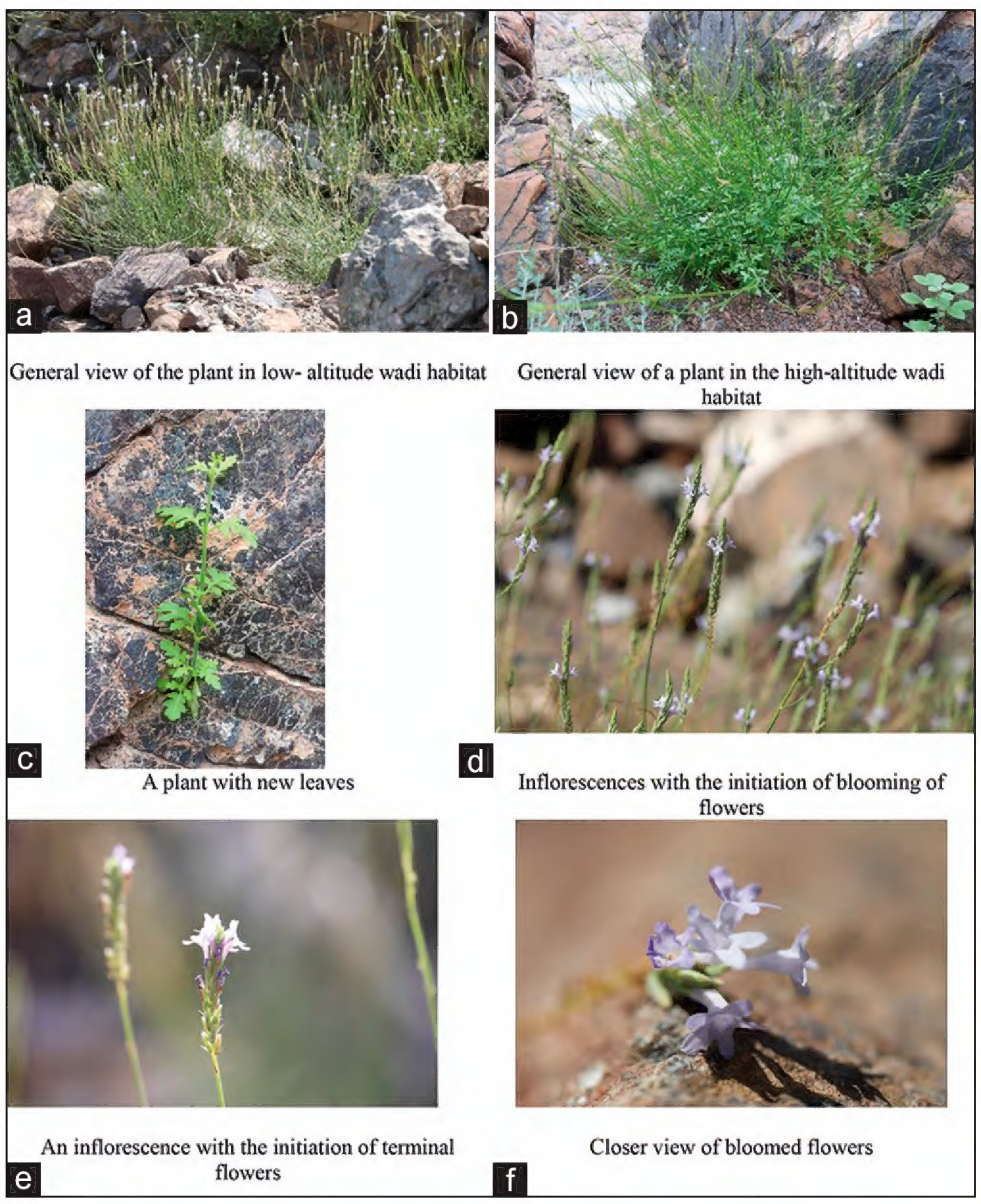


Fig 1. General map of Oman (a) and the zoomed four orange dots pointing locations (b) in four governorates where Lavandula subnuda Benth samples were collected.


a
b
General view of the plant in low- altitude wadi habitat
General view of a plant in the high-altitude wadi habitat
c
A plant with new leaves
d
Inflorescences with the initiation of blooming of flowers
An inflorescence with the initiation of terminal flowers
e
Closer view of bloomed flowers
f

Plates 1. (a-f). Images of Lavandula subnuda plants found in the wadi habitats of the Sultanate of Oman.

Ten plants were randomly selected in four sites for recording observations. Plant measurements such as plant height (m) (from the base of the plant to the highest tip of the plant) and two plant width measurements (m), one from north to south direction (N-S) (W1) and the other from east to west (E-W) (W2) were taken by the ruler. The chlorophyll contents of leaf samples were also recorded with the atLEAF CHL PLUS chlorophyll meter as atLEAF value. This chlorophyll meter has been presently used for non-destructive accurate estimates of chlorophyll in leaves (Netto et al., 2005; Zhu et al., 2012; Novichonok et al., 2016; Cahyo et al., 2020). The atLEAF CHL PLUS could measure chlorophyll content in atLEAF values (0 to 99.9) which indicate the health status of plants as the values of 35 or above refer to the grade of good or better health. Plant area (m^2) was approximated with the formula of L x B as H x (W1 + W2)/2), and the plant volume, using the formula H x L x B as HxW1xW2, mentioned above.

The edaphic characteristics of habitat and soil were briefly described for the four wadi habitats (locations), and the range of chemical contents was recorded with S1 Titan/ Tracer 5/CTX equipment of Bruker developed based on energy dispersive X-ray fluorescence (EDXRF). This equipment has been so far under application for soil mineral analysis, including soil chemical contents, in-situ or on prepared samples, powdered rock samples, "pulp", and others (Stamatis et al., 2022). Only selected macro and micro elements relevant to plant nutrition are presented here.

Essential oils extraction

Extraction of essential oil was done by using ETHOS X's advanced microwave extraction system. This

Table 1: GPS data and main soil element contents (ppm) of four wadi locations from where *Lavandula subnuda* leaf samples were collected (Edited- Included Rainfall & temperature data)

Element	Al-KhoudMo	Halban	**Najd Al-Waqba	**Al-Hayul
Longitude (E)	58.110177	58.041479	56.41579	56.31766
Latitude (N)	23.558397	23.318868	23.898453	24.3722
Altitude (m)	100.00	96	758	544
24 Year Long-term annual rainfall (mm)*				
Minimum	0.8	0.7	0.3	0.3
Maximum	16.5	19.3	20.4	20.4
Average	7.48	6.82	7.91	7.91
24 Year Long-term annual temperature (°C) *				
Minimum (Low to High)	16.7 – 30.3	14.7-31.4	11.4-30.1	11.4-30.1
Maximum (Low to High)	25.1-40.0	25.1-43.5	23.6-43.9	23.6-43.9
Average (Low to High)	23.87-33.03	23.76-35.64	21.16-35.33	21.16 – 35.33
Soil Contents	**ppm**	**ppm**	**ppm**	**ppm**
Nitrate (NO3)	121.6	91	50.0	102.3
Phosphorus (P)	36.1	30.9	14	8.7
Potassium (K)	2350	310	370	533.3
Magnesium (Mg)	1735.9	31822.5	2196.6	1766.5
Calcium (Ca)	6297.9	4329.8	2378	1411.7
Sulphur (S)	Traces	Traces	Traces	Traces
Iron (Fe)	5235.5	4355.8	4425	3339.0
Manganese (Mn)	71.5	57.2	53.1	14.4
Copper (Cu)	5.4	5	1.9	4.3
Silicon (Si)	14369.9	6997.7	15729.9	16890.8
pH	7.75	8.3	8.36	7.66
EC (Electrical Conductivity) (dS $^{-1}$)	1.322	0.881	1.035	1.406

*Rainfall and temperature data (source link https://met.gov.om/opencms/export/sites/default/dgman/en/weather-chart/historical-data/)

microwave reactor with a generator of 2.45 GHz, has been supplemented with two magnetrons with a power of 1800 W. The samples were weighed and placed inside the glass reactor (pyrex). The dry biomass of *Lavandula subnuda* (1000g) was placed in a glass container with only 2 liters of water. The glass reactor border was sealed and spread with silicone grease to avoid leaking. Then, the glass joint was connected to the glass reactor through the hole and placed on top of the microwave cavity. After that, the distillation module was connected to the glass extension tube to the joint and appropriately fastened to the external glassware support. The inlet and outlet chiller tubes were combined, and the distillation module was filled with water and connected with the glass connector. Then, the condenser was covered with a metal stopper. The chiller temperature setting was 8°C. At the end of the extraction, the essential oil was collected from the distillation module.

Essential oils analysis

GC-MS analysis was performed on a Shimadzu GC-2010 Plus Gas chromatograph, fitted with an Rtx-5MS fused silica capillary column (Restek Co., USA) (30m × 0.25mm i.d. × 0.25µm film thickness; maximum temperature, 350°C), coupled to GCMS-QP2010 ULTRA MS. Ultra-high pure helium (Grade 6.0) was a carrier gas at a constant flow of 1.11 mL/min. The injector and ion source temperatures were set at 250 °C and 275 °C, respectively, while the ionizing energy was set at 70 eV. Electron multiplier (E.M.) voltage was obtained from auto-tune. All the data were obtained within the scan range of 35-550 amu. The injected sample volume was one µl with a split ratio of 10:1. The oven temperature program was 40°C at a hold time of 1 minute and then at the rate of 4°C/min to 280°C at a hold time of 0 minutes. The run time was 61.5 minutes for each sample. The chemical compounds were identified and confirmed by comparing the spectra obtained with mass spectrum libraries (NIST 2011 v.2.3) along with arithmetic retention indices on the capillary column and relative to a homologous series of n-alkanes C7-C40. This was subsequently confirmed with published literature by comparing the calculated retention indices.

Statistical analysis

The standard errors (S.E. ±) were calculated as a part of the basic statistics from the n observations wherever found necessary in the study.

RESULTS AND DISCUSSION

Soil characters

All four sites have different wadi habitat features. On the one hand, the wadi habitats of Al-Khoud and Halban were found to be similar in features to each other, whereas on the other hand wadi features of Najd Al-Waqba and Al-Hayul were observed to be similar. Both Al-Khoud (100 m) and Halban (96 m) are at low altitudes, whereas the Najd Al-Waqba and Al-Hayul sites are at higher elevations (758m and 544m, respectively) in their respective mountains. At Wadi Al-Khoud and Wadi Halban, the foothill plains consist of mainly sandy soil, particles much coarser, differential colour from black, reddish to brownish and yellowish, and at some places pinkish coloured granite-like materials. The rocks or boulders are either fully or partly exposed with little soil with vegetation of grasses, herbs, and medium to larger shrubs, which are restricted to crevices or small depressions showing fine sediments. Both the wadis typically mainly consist of soil with more than 95% coarse sandy particles. Sites of mountainous Nazd Al-Waqba of Al-Dhahirah and Al-Hayul of Al-Buraimi were more of rocks, boulders, and stones with fewer soils attached around the crevices that are held with smaller to medium shrubs including grasses all along the ground.

The soil material of four wadis had pH in the range of 7.66 to 8.36 but varying E.C., from the lowest of 0.881 dS-1 for Halban to the highest of 1.406 dS-1 for Al-Hayul. Najd Al-Waqba had EC 1.035 dS-1, whereas Al-Khoud had 1.322 dS-1. Among the soils, the soil of Wadi Al-Khoud had the highest contents of nitrate (NO3) (121.6 ppm), phosphorous (36.1 ppm), potassium (2350 ppm), Calcium (6297.9 ppm), Iron (5235.5 ppm) and manganese (71.5 ppm) in comparison with those of other locations. However, Halban soil had the highest magnesium (31822.5 ppm), and Al-Hayul soil had the highest content of silicon (16890.8 ppm) as compared to those in other locations (Table 1).

Plant morphological characteristics

The plant characteristics of *Lavandula subnuda* were different, with higher expressions of all the attributes at the wadi Halban and Najd Al-Waqba as compared to those at Al-Khoud and Al-Hayul wadis (Table 2). Halban plants were taller (1.2 m height) with an N-S plant length of 1.9 m and E-W plant length of 1.3 m compared with Al-Hayul plants with similar stature but different in N-S and E-W lengths (0.70 m and 1.0 m, respectively). Accordingly, Halban plants had at least three times higher area (2.47 m^2 and volume (2.96 m^3) than those at wadi Al-Hayul (0.7 m^2 and 0.84 m^3, respectively). Interestingly, plants of Nazd Al-Waqba were also vigorous with the plant area and volume of 2.44 m2 and 1.833 m3, respectively, due to their longer N-S (1.25 m) and E-W (1.56 m) length. Their height at Najd Al-Waqba was less (0.94 m). (Table 2). Al-Khoud plants have lower measurements than plants from other locations (Table 2).

These measurements of plants in their wild habitats help researchers for deciding plant-to-plant distance for maximizing plant biomass yield under Good Agriculture Practice (GAP) recently (Poudeyal et al., 2019; Saha and Basak, 2020; Ierna et al., 2020) in medicinal and aromatic plants to supply to pharmaceutical industries for producing plant-based medicine molecules and drugs.

It is well known that leaf color indicative of the density of chlorophyll in leaves, which is closely associated with the status of nutrients in the plant. Hence, leaf color as a function of chlorophyll content can be considered an index for diagnosing nutrient status (Pavlovic et al., 2014; Jiménez-Lao et al., 2021; Narmilan et al., 2022). The healthy growth of plants was also manifested by their higher chlorophyll contents in terms of atLEAF value in the Lavandula plants of all locations (>30). The health features of Lavandula plants in these locations indicated that all the plants had a favorable environment in terms of water through timely rains and climatic factors like temperature and humidity for the growth and development of Lavandula plants (Table 1). The plants of Wadi Halban had the highest chlorophyll units (76.00), followed by those of Al-Hayul (65.5), Al-Waqba (54.9), and Al-Khoud (54.8).

Essential oil yield and chemical composition

In the present study, in general, a differential pattern of the essential oil yield and composition of chemical compounds was observed between the samples of locations at lower altitudes and that from higher altitudes. The quantity of essential oil recovery and the number of chemical compounds isolated were interestingly found to be higher in the samples from low altitudes than those in the samples from high altitudes. Accordingly, the essential oil yield of the Al-Khoud Wadi sample was 0.79% (w/w), and that of the Wadi Halban sample was 0.70% (w/w) (Tables 3 and 4). As against these figures, the essential oil yield of the Wadi Najd Al-Waqba sample was 0.18%(w/w), whereas that of the Wadi Al-Hayul sample was 0.15% (w/w) (Tables 5 and 6). There are no reports so far about the essential oil yield or chemical composition in respect of *Lavandula subnuda*. In the present study, the levels of the yield of essential oils of *Lavandula subnuda* obtained in plant samples from higher mountains/altitudes were found to be low as compared to those of the samples from low altitudes. These observations in terms of negative correlation between essential oil yield (%) and altitude were

Table 2: Morphological characteristics of *Lavandula subnuda* at four wadi locations of their samples' collection

Sl. No.	Characters	Al-Khoud	S.Em. (±)	Halban	S.Em. (±)	Nazd Al-Waqba	S.Em. (±)	Al-Hayul	S.Em. (±)
1.	Plant Height (m)	0.73	0.030	1.20	0.024	0.94	0.018	1.20	0.019
2.	*Plant length (N-S) (m)	0.56	0.008	1.9	0.019	1.25	0.019	0.70	0.020
3.	*Plant length (E-W) (m)	0.8	0.001	1.3	0.015	1.56	0.014	1.00	0.009
4	Approximate plant area (m^2)	0.448	0.015	2.47	0.020	2.44	0.019	0.7	0.016
5	Approximate plant volume (m^3)	0.327	0.012	2.964	0.014	1.833	0.009	0.84	0.002
6	Chlorophyll	54.8	1.089	76.00	0.758	54.9	1.002	65.5	1.003

*N-S - North-South canopy length; E-W - East-West canopy length

Table 3: Chemical composition of *Lavandual subnuda* composite leaf samples from Al-Khoud Wadi site of Al-Seeb Wilayat, Muscat governorate, Oman[1] (edited)

Sl. No.	Compound Name	R.T. (min)	Percent of essential oil (%)	KI
1	alpha.-Pinene	7.628	0.342	941
2	Camphene	8.03	0.131	1559
3	.beta.-Myrcene	9.155	0.388	1655
4	Octanal	9.48	0.101	1561
5	Limonene	10.91	1.676	984
6	Terpinolen	12.435	0.084	1035
7	1-Octen-1-ol, acetate	13.96	0.476	1105
8	Estragole	14.79	0.252	1657
9	Decanal	14.925	0.179	1173
10	Acetic acid, octyl ester	15.061	3.806	956
11	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	17.025	0.657	1426
12	Isopulegol acetate	18.555	0.255	1445
13	Copaene	19.24	0.674	1589
14	.beta.-Bourbonene	19.47	2.181	1235
15	.beta.-Elemene	19.601	4.883	1031
16	Acetic acid, decyl ester	19.895	0.458	1109
17	Isocaryophyllene	20.3	9.213	1152
18	.beta.-copaene	20.497	0.188	1526
19	5,9-Undecadien-2-one, 6,10-dimethyl-	20.921	0.176	1639
20	Humulene	21.07	1.703	1291
21	Jasminlactone	21.255	2.271	2057
22	(+)-Valencene	21.59	0.614	1022
23	D-Germacrene	21.735	42.675	1064
24	alpha.-Bulnesene	21.83	0.787	1061
25	U.I	21.885	1.201	1643
26	Germacrene B	22.05	5.485	1518
27	alpha.-Selinene	22.24	1.534	1183
28	Antioxidant 30	22.305	0.297	1019
29	Cubebol	22.44	1.271	1065
30	.delta.-Cadinene	22.58	1.490	1573
31	Kessane	22.74	2.795	997
32	Epiglobulol	22.815	0.320	1625
33	Germacrene B	23.37	1.870	1381
34	Germacren D-4-ol	23.745	2.922	1481
35	Caryophyllene oxide	23.815	0.290	1054
36	Caryophyllene oxide	23.94	1.866	1995
37	.delta.-Cedrol	25.085	0.620	976
38	Cubenol	25.13	0.794	1492
39	U.I	25.265	0.541	1911
40	U.I	25.325	0.721	1146
41	.alpha.-Cadinol	25.38	0.896	1500
42	Hedycaryol	25.68	0.278	978
43	2-Pentadecanone, 6,10,14-trimethyl-	28.835	0.638	1536
	Total		100.000	1583
Essential oil recovered	0.89mL/1000g (0.089%)			

[1]RT –Retention Time (minutes); KI- Distribution Constant; UI-Un-identified compound (not detected)

Table 4: Chemical composition of *Lavandula subnuda* composite leaf samples from Wadi Halban of Wilayat, North Barka of Al-Batinah South Governorate, Oman[I] (edited)

Sl. No.	Compound Name	R.T. (min)	Percent of essential oil (%)	KI
1	1-Octen-3-ol	8.841	0.490	939
2	Cineole	10.277	0.348	1021
3	Benzeneacetaldehyde	10.633	0.192	1036
4	.beta.-Ocimene	10.72	0.239	1039
5	1-Octanol	11.333	0.184	1065
6	Linalool	12.205	23.890	1101
7	4-t-Pentylcyclohexene	12.275	0.428	1104
8	L-trans-Pinocarveol	13.24	0.510	1144
9	U.I	13.296	0.176	1146
10	Verbenol	13.398	2.064	1150
11	U.I	13.502	0.243	1154
12	Isoborneol	13.983	6.350	1174
13	4-Terpineol	14.258	1.025	1186
14	p-Cymen-8-ol	14.478	0.442	1195
15	L-.alpha.-Terpineol	14.635	4.052	1201
16	Estragole	14.842	32.007	1209
17	2,6-Dimethyl-3,5,7-octatriene-2-ol, , E, E-	15.013	0.901	1216
18	2-Pinen-4-one	15.142	0.510	1221
19	cis-Carveol	15.329	0.345	1228
20	cis-Geraniol	15.534	0.151	1236
21	(-)-Carvone	15.989	0.185	1254
22	cis-Geraniol	16.189	0.990	1262
23	Isobornyl acetate	17.042	3.306	1295
24	Thymol	17.154	1.399	1300
25	Durenol	17.391	0.233	1310
26	4-Hydroxy-3-methylacetophenone	17.741	3.043	1324
27	Eugenol	18.775	0.772	1367
28	Damascenone	19.433	0.151	1394
29	U.I	19.603	0.208	1401
30	Jasmone	19.77	0.226	1409
31	Eugenyl methyl ether	19.86	1.840	1413
32	Isocaryophyllene	20.304	0.533	1432
33	Isogermacrene D	21.695	0.426	1493
34	.beta.-Ionone, dihydro-	22.397	0.388	1525
35	U.I	22.529	0.906	1531
36	.gamma.-Muurolene	22.597	0.198	1535
37	6-epi-shyobunol	22.661	0.450	1538
38	cis-.alpha.-Bisabolene	22.945	0.453	1551
39	Elemol	23.146	0.392	1560
40	(-)-Spathulenol	23.822	1.231	1591
41	Caryophyllene oxide	23.956	1.173	1598
42	Globulol	24.373	0.294	1618
43	Cubenol	24.581	0.390	1628
44	tau.-Cadinol	25.105	2.511	1654
45	U.I	25.29	0.409	1663
46	U.I	25.335	0.353	1665
47	alpha.-Cadinol	25.394	2.078	1668
48	Shyobunol	26.164	0.490	1706
49	U.I	28.473	0.421	1824
Total			100.000	
Essential oil recovered	0.70% w/w			

I RT –Retention Time (minutes); KI- Distribution Constant; UI-Un-identified compound (not detected)

Table 5: Chemical composition of *Lavandula subnuda* composite leaf sample from Najd Al-Waqba of Wilayat Yanqal, Al-Dhahirah governorate, Oman[1] (edited)

Sl. No.	Compound Name	R.T. (min)	Percent of essential oil (%)	KI
1	α-Pinene, (D)-	7.532	0.250	922
2	alpha.-Pinene	7.716	1.477	930
3	Camphene	8.117	0.768	943
4	Vinyl amyl carbinol	8.912	4.758	963
5	β-Myrcene	9.24	1.040	981
6	D-Limonene	10.284	4.082	1018
7	Cineole	10.358	8.036	1020
8	.beta.-Ocimene, (E)-	10.519	3.433	1036
9	cis-Sabinene hydrate	11.34	1.265	1054
10	(+)-3-Thujanol	11.928	0.495	1110
11	Linalool	12.198	6.338	1082
12	trans-2-Pinanol	12.514	0.316	1133
13	Camphor	13.473	2.576	1146
14	Verbenol	13.575	1.262	1132
15	trans-Borneol	14.039	23.465	1148
16	4-Terpinenol	14.325	18.734	1162
17	.alpha.-Terpineol	14.672	3.620	1172
18	Verbenone	15.181	3.367	1170
19	D-Carvone	16.054	0.715	1194
20	L-bornyl acetate	17.105	0.651	1273
21	Jasmone	19.832	0.885	1359
22	DDA (Antioxidant)	22.066	3.788	1496
23	Kessane	22.822	3.568	1536
24	Palmitic acid, methyl ester	30.385	3.152	1908
25	Elaidic acid, methyl ester	33.373	0.946	2084
26	Methyl stearate	33.787	0.946	2133
Total			100.000	
Essential oil recovered	0.1g/550g (0.0181%)			

[1]RT –Retention Time (minutes); KI- Distribution Constant

Table 6: Chemical composition of *Lavandula subnuda* composite leaf sample from Wadi Al-Hayul of Wilayat Mahdha, Al-Buraimi Governorate, Oman[1] (edited)

Sl. No.	Compound Name	R.T. (min)	Percent of essential oil (%)	KI
1	alpha.-Bourbonene	23.769	1.945	1384
2	.beta.-Elemene, (-)-	23.991	10.618	1391
3	beta.-cis-Caryophyllene	24.850	13.684	1419
4	.alpha.-Caryophyllene	25.892	3.755	1454
5	Germacrene D	26.723	4.969	1481
6	beta.-Eudesmene	26.883	5.604	1487
7	6-epi-shyobunol	26.968	5.085	1489
8	delta.-Guaiene	27.15	6.043	1495
9	Germacrene D	27.705	0.988	1514
10	U.I	27.972	1.486	1524
11	Kessane	28.108	18.603	1528
12	U.I	28.195	1.539	1532
13	Caryophyllene oxide	29.707	1.285	1584
14	U.I	30.553	1.552	1614
15	.gamma.-Eudesmole	31.349	7.993	1644
16	Bulnesol	31.533	5.734	1650
17	U.I	31.686	3.183	1656
18	U.I	32.482	3.949	1685
19	alpha.-Phellandrene, dimer	35.315	1.987	1794
			100.000	
Essential oil recovered	0.093g/606.2g of sample (0.0153%)			

[1] RT –Retention Time (minutes); KI- Distribution Constant; UI-Un-identified compound (not detected)

also made by Dizajeykan et al. (2016) in *Thymus pubescence* and Nejad Ale Omrani et al. (2019) in *Oleveria decumbens*. This is attributed to variations in genetic adaptation response of plants to specific habitats for the synthesis of metabolites (polar or volatile compounds) through variation in metabolic pathways (Seyis et al., 2020). Further, essential oil recovery levels of the present studies can be comparable to that of other species of *Lavandula* in other previous studies. Messsaoud et al. (2011) reported essential oil from 0.21 % (*L. coronopifolia*) to 0.26% (*L. multifida*) and 0.86% (L. stoechas). Jianu et al.(2013) reported 1.13 % (w/w), whereas Babu et al. (2016) reported essential oil % of 0.6 to 1.8% in L. angustifolia from supercritical CO_2 extraction (SCE) and Salata et al. (2020) found essential oil from 0.54 mg100 g-1 to 1.13 mg100 g-1, which were very low in their experiment on the effect of irrigation and drying method on essential oil and its chemical composition in *L. angustifolia*. In *L. dentata*, the essential oil content of flowers and leaves was reported to be 0.0086% and 0.0066, respectively, by Touati et al. (2011).

Similarly, in the present study, a differential pattern of the composition of chemical compounds was found between the samples of locations at low altitudes and that from high altitudes. A higher number of chemical compounds was found at lower altitudes (<100 m; >40 compounds) than at higher altitudes (544-758 m; <26 compounds). The plausible explanation for this fact is yet to be ascertained by further investigations in terms changes in number of chemical contents that are produced through changes in biochemical pathways due to altitudinal changes (temperatures). However, these observations are in line with the results of Salata et al. (2020), who found a negative correlation between essential oil yield and its contents with altitude in their study on yield, essential oil content, and quality performance as affected by supplementary irrigation and drying methods. Similarly, the chemical composition of essential oils of medicinal plants is strongly influenced on environmental conditions involving both edaphic and climatic factors of

the locations (Selmar and Kleinwachter, 2013; Radwan et al., 2017; Karalija et al., 2022).

In the present study, of the locations of lower altitudes, in the case of the essential oil of *Lavandula subnuda* of Wadi Al-Khoud, of the total 43 chemical compounds found, the highest of 40 chemical compounds were identified while three were not identified (UI), which in total, represent 100% of essential oil in the range from 0.084% (terpinolene) to 39.573% (d-germacrene (Table 3; Fig. 2).

In the case of Wadi Halban essential oil, however, there were a total of 49 compounds found, of which only 42 chemical compounds were identified, and the remaining seven were unidentified. These, in total, represented 100 % of essential oil in the range from 0.176 (% unidentified compound) to 32.007% (Estragol) Table 4: Fig. 3).

Contrarily, from the locations of higher altitudes, all 26 chemical compounds were identified from the essential oil of Lavandula of Wadi Najd Al-Wakba, which in total represented 100% of essential oil in the range from 0.316% (trans 2-Pinanol) to 23.465% (trans-Borneol) (Table 5; Fig. 4). However, in the case of Wadi Al-Hayul essential oil, there were a least of 19 compounds found, of which only 14 chemical compounds were identified, and the

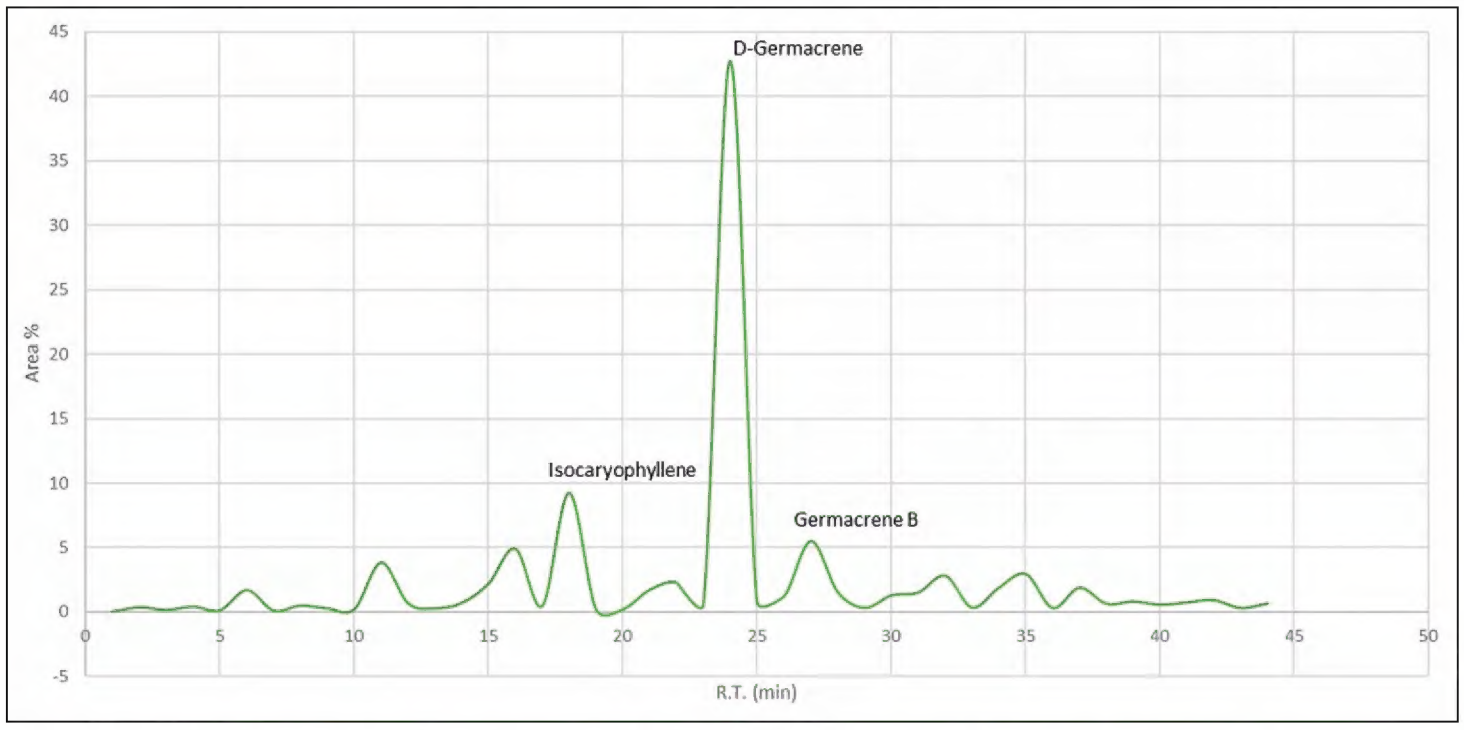


Fig 2. Chromatograms of the volatile fraction of Lavandula subnuda determined by means of HS–GC–MS for composite leaf sample from Wadi Al-Khoud of Al-Seeb Wilayat, Muscat governorate, Oman; (i.e., most abundant and/or best separated) peaks are labeled according to the numbering attributed to them in Table 3.

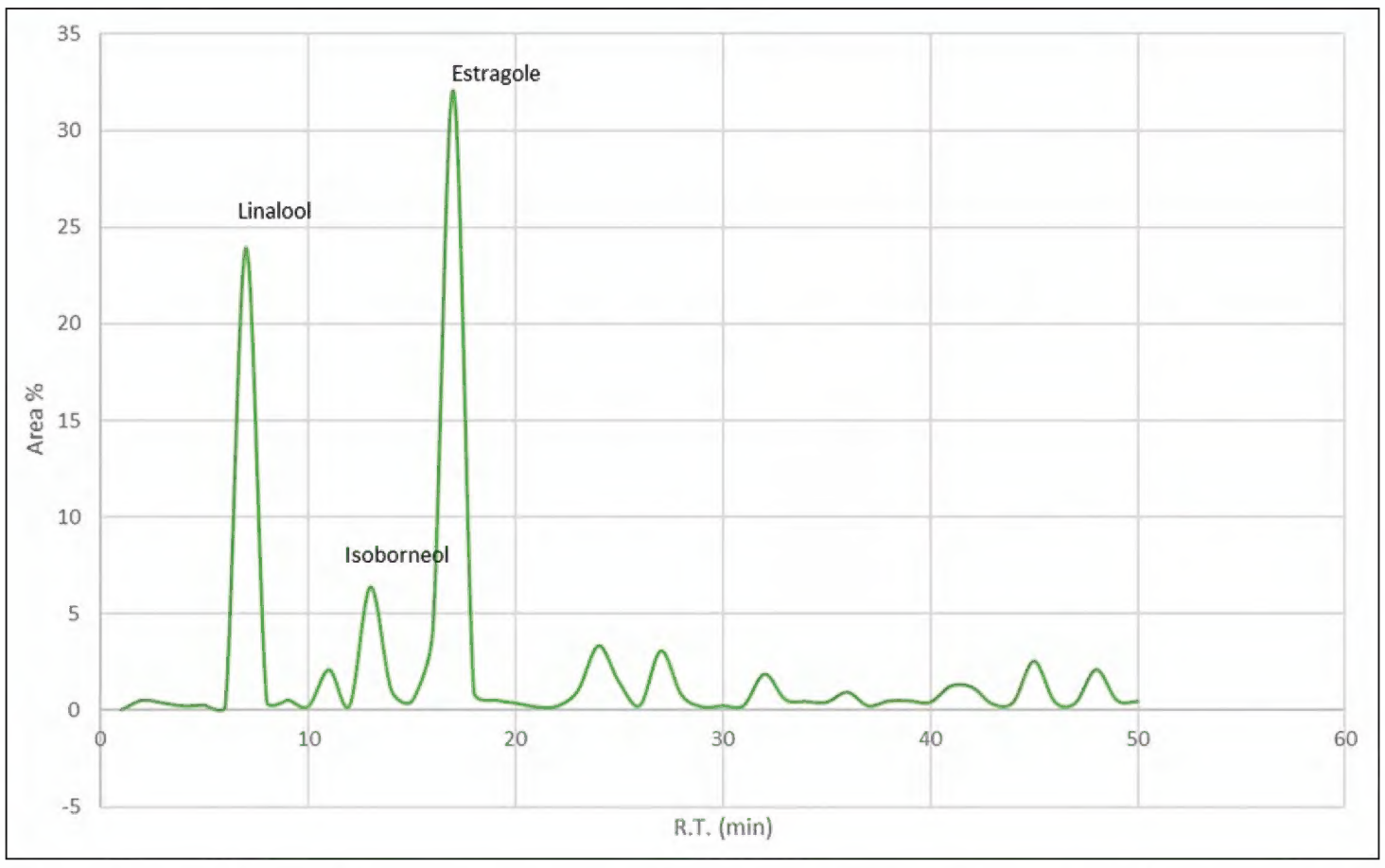


Fig 3. Chromatograms of the volatile fraction of Lavandula subnuda determined by means of HS–GC–MS for composite leaf sample from Wadi Halban of Al-Barka Wilayat, South Batinah Governorate, Oman; (i.e., most abundant and/or best separated) peaks are labeled according to the numbering attributed to them in Table 4.

remaining five, unidentified. These, in total, represented 100 % of essential oil in the range from 0.988 (% unknown) to 18.603% (Kessane) (Table 6: Fig. 5).

Interestingly, when we compared the common chemical compounds found among the four locations in combinations (pairs), it was observed that these pairs were common with 1 compound to 5 compounds (Table 7) with either similar and differential percent of oil (Tables 3 to 6). Thus, the essential oils of Al-Khoud and Halban (apha-Cardinol, Caryophyllene oxide, Cubenol, Estragole and Isocaryophyllene) and that of Halban and Najd Al-Waqba (4-Terpinol, Cineole, Jasmone, Linalool, Verbenol) were found common with five chemical compounds whereas the essential oils of Al-Khoud and Al-Hayul (D-Germacrene and Kessane) and that of Halban and Al-Hayul (6-epi-shyobunol and Caryophyllene oxide) were common in two chemical compounds. However, the essential oils of Al-Khoud and Najd Al-Waqba were common with three compounds (alpha-Pinene, Camphene and Kessane), whereas those of Najd Al-Waqba and Al-Hayul were common in only one chemical compound (Kessane) (Table 7).

Further, in the essential oils of all four locations, the contribution of the top ten chemical compounds was found to vary between 78.101% and 82.282%. Of the top ten higher contents of the Al-Khoud essential oil,

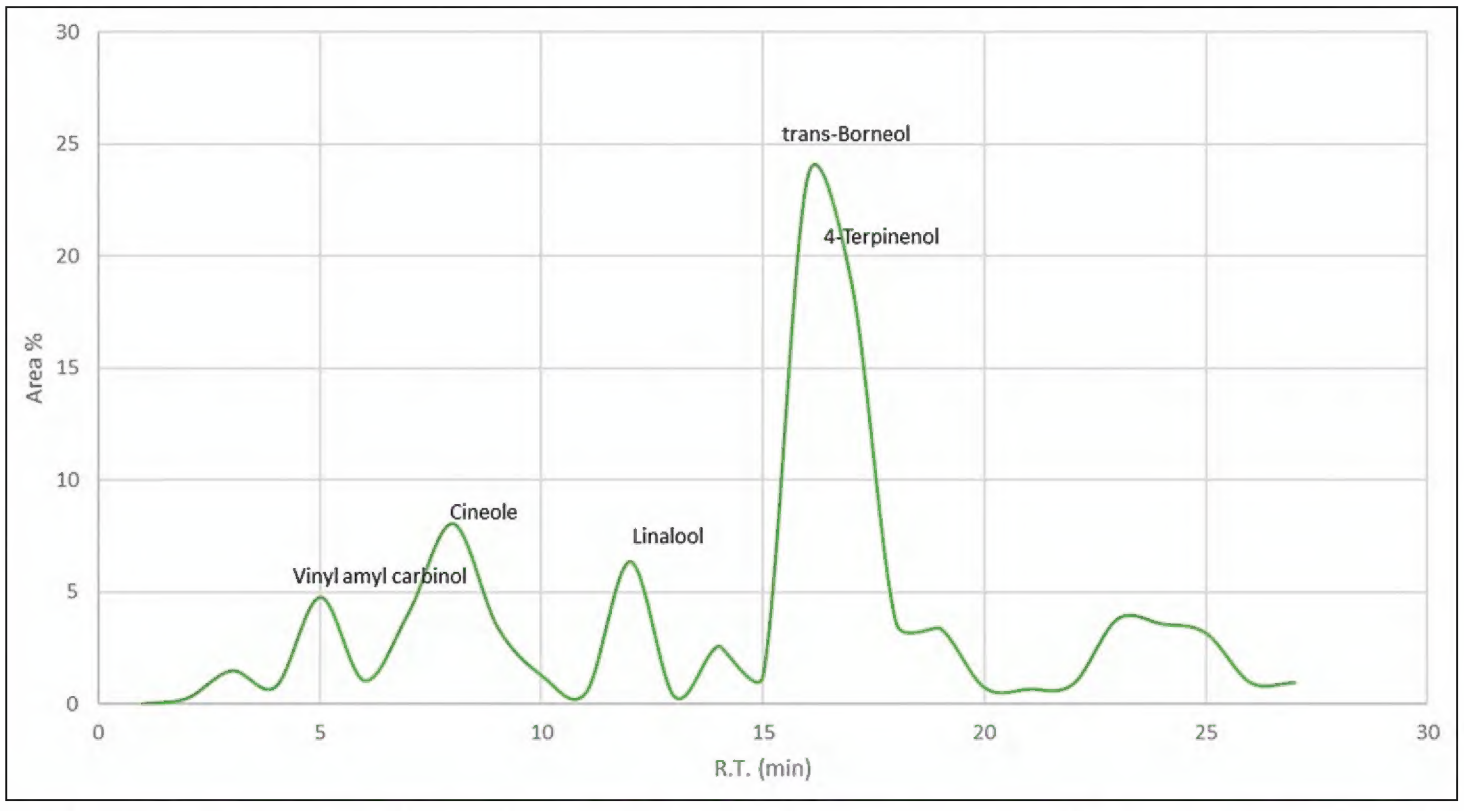


Fig 4. Chromatograms of the volatile fraction of Lavandula subnuda determined by means of HS–GC–MS for composite leaf sample from Najd Al-Waqba of Wilayat Yanqal, Al-Dhahirah governorate, Oman; (i.e., most abundant and/or best separated) peaks are labeled according to the numbering attributed to them in Table 5.

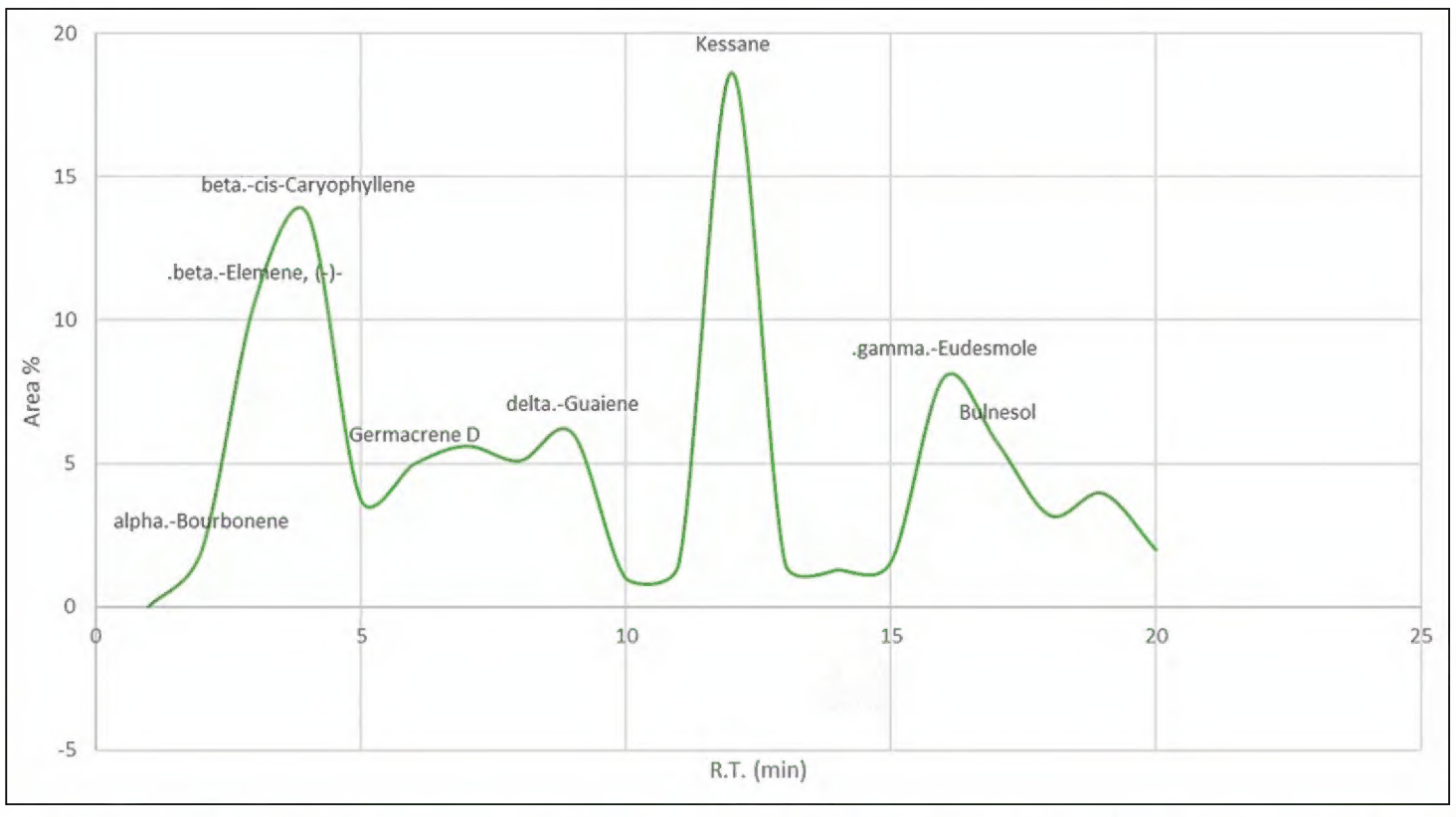


Fig 5. Chromatograms of the volatile fraction of Lavandula subnuda determined by means of HS–GC–MS for composite leaf sample from Wadi Al-Hayul of Wilayat Mahdha, Al-Buraimi Governorate, Oman; (i.e., most abundant and/or best separated) peaks are labeled according to the numbering attributed to them in Table 6.

D-Germacene contributed the most (42.675%), followed by Isocaryophyllene (9.213%), Germacrene B (5.485%), and beta-Elemene (4.883%) The remaining six compounds, were with a lower degree of concentration (<5 %; Table 8).

Similarly, of the top ten higher contents of the Halban essential oil, Estragole occupied the highest of 32.007%, followed by Linalool (23.890%) and Isoborneol (6.350) while the remaining six compounds were with a lower degree of concentration (<5 %; Table 9).

Of the top ten higher contents of the essential oil of Najd Al-Waqba, trans-Borneol ranked the highest in the concentration (23.465%), followed by 4-Terpinenol (18.734 %), Cineole (8.036%) and Linalool (6.338%) while the remaining six compounds were with a lower degree of concentration (<5 %; Table 10).

Finally, of the top ten higher contents of the Al-Hayul essential oil, Kessane occupied the highest concentration (18.603%), followed by beta–cis-Caryophyllene (13.684%), beta –Elemene (10.618%), gama Eudesmol (7.993%) and delta Guaiene (6.043 while the remaining five compounds were with a lower degree of concentration (<6 %; Table 10). (<5 %; Table 11).

This is the first report on the essential oil yield (%) and chemical composition of *Lavandula subnuda*. However, there are many studies conducted earlier on other different species of lavender like *Lavandula angustifolia* (Jianu et al., 2013; Smigielski et al., 2009 & 2013; Pokajewicz et al., 2021), *Lavandula coronopifolia* (Messaoud et al., 2011), *Lavandula multifeda* (Messaoud et al., 2012), *Lavandula stoechas* (Messaoud et al., 2012), *Lavandula dentata* (Imelouane et al., 2010; Touati et al., 2011; Justus et al., 2018) etc. Interestingly, the results of our study on the chemical composition of essential oils of *Lavandula*

Table 7: Number of chemical contents of *Lavandula subnuda*, which are common in the plant samples of four locations of Oman

Locations	Halban (49)*	Najd Al-Waqba (26)	Al-Hayul (19)
Al-Khoud (43)	5 alpha.-Cadinol Caryophyllene oxide Cubenol Estragole Isocaryophyllene	3 alpha-Pinene Camphene Kessane	2 D-Germacrene Kessane
Halban (49)		5 4-Terpineol Cineole Jasmone Linalool Verbenol	2 6-epi-shyobunol Caryophyllene oxide
Najd Al-Waqba (26)			1 Kessane

*Total number of chemical contents identified in each location

Table 8: Top 10 chemical compounds of *Lavandula subnuda* composite sample from Wadi Al-Khoud of Al-Seeb Wilayat, Muscat governorate, Oman[I] (Edited)

Sl.No.	Compound Name	R.T. (min)	Percent of Essential oil (%)	KI
1	D-Germacrene	21.735	42.675	1501
2	Isocaryophyllene	20.3	9.213	1396
3	Germacrene B	22.05	5.485	1534
4	.beta.-Elemene	19.601	4.883	1387
5	Acetic acid, octyl ester	15.061	3.806	1191
6	Germacren D-4-ol	23.745	2.922	1576
7	Kessane	22.74	2.795	1522
8	Jasminlactone	21.255	2.271	1442
9	.beta.-Bourbonene	19.47	2.181	1386
10	Germacrene B	23.37	1.870	1534
	Total		78.101	

I RT –Retention Time (minutes); KI- Distribution Constant

Table 9: Top 10 chemical compounds of *Lavandula subnuda* composite sample from Wadi Halban of wilayat Barka, South Batinah governorate, Oman[I] (Edited)

Sl.No.	Compound Name	R.T. (min)	Percent of Essential oil (%)	KI
1	Estragole	14.842	32.007	1186
2	Linalool	12.205	23.890	1081
3	Isoborneol	13.983	6.350	1174
4	L-.alpha.-Terpineol	14.635	4.052	1192
5	Isobornyl acetate	17.042	3.306	1268
6	4-Hydroxy-3-methylacetophenone	17.741	3.043	1292
7	tau.-Cadinol	25.105	2.511	1637
8	alpha.-Cadinol	25.394	2.078	1641
9	Verbenol	13.398	2.064	1132
10	Eugenyl methyl ether	19.86	1.840	1378
	Total		81.141	

I RT –Retention Time (minutes); KI- Distribution Constant

Table 10: Top 10 chemical compounds of *Lavandula subnuda* composite sample from Wadi Najd Al-Waqba of Wilayat Yanqal, Al-Dhahirah governorate, Oman[I] (Edited)

Sl.No.	Compound Name	R.T. (min)	Percent of Essential oil (%)	KI
1	trans-Borneol	14.039	23.465	1148
2	4-Terpinenol	14.325	18.734	1162
3	Cineole	10.358	8.036	1020
4	Linalool	12.198	6.338	1082
5	Vinyl amyl carbinol	8.912	4.758	963
6	D-Limonene	10.284	4.082	1018
7	DDA (Antioxidant)	22.066	3.788	1496
8	alpha.-Terpineol	14.672	3.620	1172
9	Kessane	22.822	3.568	1536
10	beta.-Ocimene, (E)-	10.519	3.433	1036
	Total		79.822	

I RT –Retention Time (minutes); KI- Distribution Constant

Table 11: Top 10 chemical compounds of *Salvia macilenta* composite sample from wadi Al-Hayul of Wilayat Mahdha, Al-Buraimi Governorate, Oman[1] (Edited)

Sl.No.	Compound Name	R.T. (min)	Percent of Essential oil (%)	KI
1	Kessane	28.108	18.603	1536
2	beta.-cis-Caryophyllene	24.85	13.684	1396
3	.beta.-Elemene, (-)-	23.991	10.618	1387
4	.gamma.-Eudesmole	31.349	7.993	1620
5	delta.-Guaiene	27.15	6.043	1508
6	Bulnesol	31.533	5.734	1652
7	beta.-Eudesmene	26.883	5.604	1478
8	6-epi-shyobunol	26.968	5.085	1505
9	Germacrene D	26.723	4.969	1480
10	U.I	32.482	3.949	
	Total		82.282	

1 RT –Retention Time (minutes); KI- Distribution Constant; UI-Un-identified compound (not detected)

subnuda from four diverse locations indicated that the chemical contents of essential oils were different from each other. This is attributed to genetic factors like the genotype or genetic constitution, factors like the chemical profile of natural products, and environmental factors like climatic conditions, seasonal and geographic conditions, edaphic factors, elevation/altitude, and topography, etc., and their interaction effects (Skoula et al., 2000; Couladis et al., 2001; Ebrahimi et al., 2008; Stanojevic et al., 2011and Fattahi et al., 2016).

Regarding the chemical compounds of essential oils of *Lavandula subnuda* from four diverse locations, we found that eight chemical compounds such as D-Germacrene (42.675%) in the sample from Wadi Al-Khoud, Estragole (32.007%), and Linalool (23.890%) in that of Wadi Halban, trans-Borneol (23.465%) and 4-Terpinenol (18.734%) in that of Wadi Nejd Al-Waqba, and Kessane (18.603%), beta.-cis-Caryophyllene (13.684%) and beta-Elemene (10.618%) in that of Wadi Al-Hayul, were in higher proportion than other compounds. However, in earlier studies, of these seven compounds, only two compounds, namely Caryophyllene (24.1% of Jianu et al., 2013) and Linalool (30.6% of Smigielski et al., 2013 and 46.7% of Pokajewicz et al., 2021 in *Lavandula angustifolia* and 49.9% of Ozcan et al., 2018 in *Lavandula dentata*) were found in higher proportion.

Among the eight medicinal compounds, Kessane is still under research and thus has no specific use as of now. Linalool is associated with the perfumery industry (Elsharif et al.,2015). However, the rest all the six compounds are more relevant for medicinal use with their anti-bacterial and anti-fungal or anti-microbial properties. Given the merits of these six compounds in *Lavendula subnuda*, as mentioned above, it is recommended that this medicinal plant species be explored for the isolation of plant-based molecules for their safe use for the welfare of humanity. The isolation of molecules is commercially feasible on a large scale by producing large biomass of the plants by growing *Lavandula subnuda* in the field with Good Agriculture Practice (GAP) formulated using specific plant characteristics like approximated plant area (m^2) and plant volume (m^3) for optimum plant density with irrigation scheduling and fertilizer management. The low levels of essential oil extraction observed in the present study can be enhanced through elicitation treatments by using elicitors such as Salicylic acid, as reported recently in *Ocimum gratissimum* L. (Alvarenga et al. (2021).

CONCLUSIONS

There exist differential expression of *Lavandula subnuda* in terms of the chemical composition depending on environmental factors, which can be considered in its further exploration through cultivation, extraction of essential oil and isolation of the most valuable molecules like linalool in aromatic industries and D-Germacrene, Estragole, trans-Borneol, 4-Terpinenol, beta.-cis-Caryophyllene and beta-Elemene in pharmaceutical industries.

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Authors contribution

Saleem K Nadaf planned the investigation, statistically analyzed the data and written the original draft and modified/revised based on inputs of all other authors. Jamal N. Al-Sabahi and Ali H Al-Lawati devised and helped in the chemical analysis and collection & preservation of plant samples, respectively. Almandhar R. Al-Mamari and Abdulaziz A. Al-Mawali collected seed and plant samples. Fatima A. Al-Kindi, Houda K. Al-Ruqaisi and Ahmed S. Al-Ghafri helped in extraction of essential oil and its chemical analyses. Amina Al-Farsi confirmed the specimens as that of Lavandula subnuda Boiss. and Nadiya A Al-Saady arranged for funds and supervised the investigation.

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